PATENT
Attorney Docket No. 213721

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Feussner et al.

Art Unit: Unassigned

Application No. Unassigned
(U.S. National Phase of PCT/EP00/02545)

Examiner: Unassigned

Filed: September 28, 2001

For: LINOLEATE- AND LINOLENATE-LIPOXYGENASE MUTANTS

AMENDMENTS TO CLAIMS
MADE VIA PRELIMINARY AMENDMENT

[1. Process for the manufacture of a plant lipoxygenase with altered position specificity, including the step

- submission of one or more amino-acids in a wild-type lipoxygenase.]

[2. Process according to Claim 1, characterized in that the one or more amino-acid substitution(s) take place in the region of amino-acid position 527 to 536 and/or 593 to 602 of the lipoxygenase from *Cucumus sativus* or a corresponding position in a lipoxygenase from another variety of plant.]

[3. Process according to Claim 2, characterized in that the substitution takes place at position 531 and/or 597 of the lipoxygenase from *Cucumis sativus* or a corresponding position in a lipoxygenase from another plant.]

[4. Process according to Claim 3, characterized in that the substitution at position 531 puts into place a Phe- or His- residue and/or at position 597 a Val- or Phe- residue in the mutant.]

[5. Process according to Claim 4, characterized in that the substitution at position 531 represents a Val-- > Phe- and/or at position 597 a His-- > Val- substitution.]

[6. Process according to one of Claims 1-5, characterized in that the amino-acid substitution is inserted by directed mutagenesis.]

[7. Lipoxygenase, obtainable through a process according to one of the Claims 1-6.]

[8. Nucleic acid which codes for a lipoxygenase according to Claim 7.]

[9. Vector containing a nucleic acid according to Claim 8.]

[10. Cell containing a nucleic acid according to claim 8 and/or a vector according to Claim 9.]

[11. Plant of part of a plant including a host cell according to Claim 10.]

[12. Process for the manufacture of 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid, including the step

- Conversion of γ-linolenic acid with a lipoxygenase according to Claim 7.]

[13. Use of a lipoxygenase according to Claim 7 for the manufacture of 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid.]

[14. γ-linolenic acid derivative containing a hydroperoxy group or a hydroxy group at position 6.]

15. A method of making a plant lipoxygenase with altered position specificity, which method comprises substituting one or more amino acids in a wild-type plant lipoxygenase, whereupon a plant lipoxygenase with altered position specificity is obtained.

16. The method according to claim 15, in which the lipoxygenase is from *Cucumis sativus* and the one or more amino acids are in the region of amino acid position 527 to 536 and/or 593 to 602 of the lipoxygenase or the lipoxygenase is from another variety of plant and the one or more amino acids are in the region of the lipoxygenase corresponding to amino acid position 527 to 536 and/or 593 to 602 of the lipoxygenase from *Cucumis sativus*.

17. The method according to claim 16, characterized in that the one or more amino acids are at amino acid position 531 and/or 597 of the lipoxygenase from *Cucumis sativus* or a corresponding position in a lipoxygenase from another plant.

18. The method according to claim 17, characterized in that the amino acid at position 531 is substituted with a Phe- or His- residue and/or the amino acid at position 597 is substituted with a Val- or Phe- residue.

19. The method according to claim 18, characterized in that the amino acid at position 531 is a Val- and is substituted with a Phe- and/or the amino acid at position 597 is a His- and is substituted with a Val-.

20. The method according to claim 15, characterized in that the substituting is by directed mutagenesis.

21. A lipoxygenase obtained in accordance with the method of claim 15.

22. A lipoxygenase obtained in accordance with the method of claim 16.

23. A lipoxygenase obtained in accordance with the method of claim 17.

24. A lipoxygenase obtained in accordance with the method of claim 18.

25. A lipoxygenase obtained in accordance with the method of claim 19.

26. An isolated nucleic acid molecule which consists essentially of a nucleotide sequence encoding the lipoxygenase of claim 21, optionally in the form of a vector.

27. An isolated nucleic acid molecule which consists essentially of a nucleotide sequence encoding the lipoxygenase of claim 22, optionally in the form of a vector.

28. An isolated nucleic acid molecule which consists essentially of a nucleotide sequence encoding the lipoxygenase of claim 23, optionally in the form of a vector.

29. An isolated nucleic acid molecule which consists essentially of a nucleotide sequence encoding the lipoxygenase of claim 24, optionally in the form of a vector.

30. An isolated nucleic acid molecule which consists essentially of a nucleotide sequence encoding the lipoxygenase of claim 25, optionally in the form of a vector.

31. A cell comprising the isolated nucleic acid molecule of claim 26.

32. A cell comprising the isolated nucleic acid molecule of claim 27.

33. A cell comprising the isolated nucleic acid molecule of claim 28.

34. A cell comprising the isolated nucleic acid molecule of claim 29.

35. A cell comprising the isolated nucleic acid molecule of claim 30.

36. A plant or a plant part comprising the cell of claim 31.

37. A plant or a plant part comprising the cell of claim 32.

38. A plant or a plant part comprising the cell of claim 33.

39. A plant or a plant part comprising the cell of claim 34.

40. A plant or a plant part comprising the cell of claim 35.

41. A method of making 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid, which method comprises incubating γ-linolenic acid with the lipoxygenase of claim 21 under suitable conditions, whereupon 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid is obtained.

42. A method of making 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid, which method comprises incubating γ-linolenic acid with the lipoxygenase of claim 22 under suitable conditions, whereupon 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid is obtained.

43. A method of making 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid, which method comprises incubating γ-linolenic acid with the lipoxygenase of claim 23 under suitable conditions, whereupon 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid is obtained.

44. A method of making 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid, which method comprises incubating γ-linolenic acid with the lipoxygenase of claim 24 under suitable conditions, whereupon 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid is obtained.

45. A method of making 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid, which method comprises incubating γ-linolenic acid with the lipoxygenase of claim 25 under suitable conditions, whereupon 6-, 9- and/or 6,9-hydroperoxy-γ-linolenic acid is obtained.

46. A derivative of γ-linolenic acid containing a hydroperoxy group or a hydroxy group at postion 6.